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Lectera, a new genus of the *Plectosphaerellaceae* for the legume pathogen *Volutella colletotrichoides*

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Abstract

Volutella colletotrichoides is shown to belong to the *Plectosphaerellaceae* rather than the *Hypocreales* where other species of that genus reside. The new genus *Lectera* is described for *V. colletotrichoides*, and for a further, previously undescribed species with slightly longer conidia and differences in rDNA ITS and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) sequences. *Lectera* species are found to be soil-dwelling organisms associated particularly with species of *Fabaceae*, and *L. colletotrichoides* is demonstrated to be a pathogen of *Cicer arietinum* and other grain legumes.

Key words

Systematics, plant pathogenic fungi, phylogeny, *Cicer arietinum*

Introduction

Volutella colletotrichoides was first described from diseased alfalfa and other forage legumes collected from Iowa, USA (Chilton 1954), evident as pink acervular dark-setose fruit bodies erumpent from stems and leaves. It was compared with four other species of *Volutella* known to be associated with legumes, and distinguished by its acervular setae that are dark rather than hyaline, and by the size of its conidia. The dark setae and

relatively large conidia invited comparison with *Colletotrichum* and led to choice of the specific epithet. A collection with especially numerous setae was given varietal status as *V. colletotrichoides* var. *setosa.*

The pathogenicity of *Volutella colletotrichoides* was demonstrated by observations of naturally infected plants in the glasshouse and in the field, and also by infection experiments (Chilton 1954). The original samples derived from stem cuttings of *Medicago sativa* (alfalfa), *Trifolium pratense* (red clover) and *Lotus corniculatus* (bird's foot trefoil), with *Medicago* plants proving the most vulnerable to infection, and were reproduced in plant samples transferred to Lincoln, Nebraska. Periodic observations over the following two years showed sparse and erratic occurrence of the pathogen, suggesting that the fungus is not a virulent pathogen, at least in those local conditions. Chilton found that inoculations on mature alfalfa and red clover plants produced petiole girdling and girdling of the stem tips, causing a blighted appearance in severely infected plants. Infection of mature stem tissues produced elliptical sunken lesions to a limited extent. No evidence of systemic invasion of the plant was found although stem infections gradually enlarged to involve much of the plant under high moisture conditions.

Host range experiments (Chilton 1954) provided evidence that *Trifolium hybridum* and *Medicago falcata* were susceptible to infection, in addition to the three plant species on which the original observations were made. In contrast, *Melilotus alba* and *Glycine max* were not infected under similar experimental conditions. As the plants originally infected were of foreign origin (Chilton did not specify from where) and the occurrence of the pathogen was highly localized, it seems likely that *Volutella colletotrichoides* was a pathogen introduced into the USA that did not become established, rather than a native species. There are no further records of the species from the USA, and no material in the national fungus collection **BPI** (Farr and Rossman 2011).

Published reports of *Volutella colletotrichoides* from other regions are sparse. It was reported from *Glycine max* in Ethiopia by Mengistu and Sinclair (1979), from the same host (as *G. ussuriensis*) in Democratic Republic of the Congo by Lenné (1990), and on *Senna sophera* from India (Lenné 1990). Eken et al. (2002) showed the fungus to be present on alfalfa in Turkey. A sequence from an unlocalised sample on *Viola* sp. was submitted to GenBank (http://www.ncbi.nlm.nih.gov/nuccore/AJ301962.1) by Hagedorn (unpubl., 2002), and cultures from alfalfa from Turkey (reported above) and South Africa are stored in the CBS culture collection (http://www.cbs.knaw.nl/). The plant associates given above do not necessarily imply a biological relationship.

In the winter of 1988, a field survey was carried out for diseases of chickpea (*Cicer arietinum*) in the vicinity of Salheia, at that time a newly reclaimed region between the Nile Delta and the Suez Canal (Ismaelia) in northern Egypt. There, blighted chickpea plants were observed and collected, with the causal organism initially assumed to be the common chickpea pathogen *Ascochyta rabiei*. The macroscopic visible symptoms were circular or elongated dark brown to black anthracnose-like lesions approximately one centimeter long on the lower stem parts in addition to partly chlorotic leaves. The

causal organism was isolated and sent to the Danish Government Institute of Seed Pathology for Developing Countries (DGISP), and designated as DGISP 271. Later the diagnostic studies were transferred to The Danish Veterinary and Agricultural University. Here the organism was registered as CP 2035. After an extended period of analysis using both morphological and molecular methods with multiple collaborators, the affinities of CP 2035 have finally been confirmed to be with *V. colletotrichoides*.

As Chilton (1954) had observed, we found that *Volutella colletotrichoides* differed in a number of morphological characteristics from other *Volutella* species. As molecular analysis has confirmed this separation, we therefore describe the new genus *Lectera* for *V. colletotrichoides* and a similar, closely related species.

Materials and methods

Isolates were retrieved from storage in the CABI Genetic Resources Collection (see Table 1). For morphological analysis, strains were grown at 25°C for 7 d on PCA and PDA media (Smith and Onions 1994). Observations were made using microscope preparations in water and lactic acid, with measurements made from slides mounted in lactic acid.

Infection studies were carried out, using chickpea cultivar Family 88 as well as certain other species of *Fabaceae* as hosts: *Vicia faba*, *Glycine max*, *Pisum sativum*, *Vigna unguiculata*, and *Phaseolus vulgaris*. The inoculum was produced as above. Stems and leaves were inoculated without prior wounding using a spore suspension produced by flooding four week old cultures with 10 ml sterile distilled water. The conidial concentration was adjusted to 2×10^5 ml^{-1} and finally one drop of Tween 20 (as detergent) was added. Symptoms were observed two weeks after inoculation.

For the molecular analysis, strains were grown on malt extract (MADW) agar plates (Smith and Onions 1994) at 28°C for 5–7 days. The isolates were subcultured onto fresh MADW plates and incubated at 28°C for 7–10 days prior to DNA extraction.

Total genomic DNA was obtained from a small loopful (1 µl) of each strain using a proprietary complex DNA release solution (microLYSIS®–PLUS; Microzone Ltd, UK) in accordance with manufacturer's instructions. The thermal cycler lysis profile was: 15 min at 65°C, 2 min at 96°C, 4 min at 65°C, 1 min at 96°C, 1 min at 65°C, 30 s at 96°C and hold at 20°C.

Partial ribosomal RNA gene clusters (part of 18S small subunit RNA gene, internal transcribed spacer 1 (ITS1), 5.8S ribosomal RNA gene, internal transcribed spacer 2 (ITS2), part of 28S large subunit ribosomal RNA gene) were amplified by polymerase chain reaction (PCR) using primer set TW81 (fwd): 5'–GTTTCCGTAGGTGAACCTGC–3' & AB28 (rev): 5`–ATATGCTTAAGTTCAGCGGGT–3' (Curran et al. 1994; Sigma Genosys, UK). Part of the glyceraldehyde 3-phosphate dehydrogenase gene was amplified using the primers GDF: 5'-GCCGTCAACGACCCCTTCATTGA-3' & GDR: 5'-GGGTGGAGTCGTACTTGAGCATGT-3' (Prihastuti et al.

Table 1. Newly sequenced strains in this study

Reference no.	Species	Host/substratum, geographical origin	ITS	GAPDH
IMI 185380	*Acremonium nepalense*	*Pinus* sp. soil, Nepal	JQ647430	
IMI 337226	*Bionectria coronata*	*Serjania* sp., Venezuela	JQ700569	
IMI 46339	*Gibellulopsis nigrescens*	Soil, UK	JQ647441	JQ724142
IMI 118380	*Gibellulopsis nigrescens*	*Beta vulgaris*, Canada	JQ647440	JQ724134
IMI 265740	*Lectera colletotrichoides*	Soil, Brazil	JQ647449	JQ724136
IMI 303685	*Lectera colletotrichoides*	*Capsicum annuum*, Morocco	JQ647450	JQ724140
IMI 332702	*Lectera colletotrichoides*	*Cicer arietinum*, Egypt	JQ647428	
IMI 366179	*Lectera colletotrichoides*	*Phaseolus vulgaris*, Ethiopia	JQ693168	
IMI 368065	*Lectera colletotrichoides*	*Xanthium spinosum*, Argentina	JQ647451	JQ724141
IMI 181698	*Lectera longa*	*Triticum* sp., Australia	JQ647448	JQ724135
IMI 312228	*Plectosphaerella cucumerina*	*Daucus carota*, Denmark	JQ647434	JQ724139
IMI 49467b	*Pseudonectria buxi*	*Buxus sempervirens*, UK	JQ693165	
IMI 61338	*Pseudonectria buxi*	*Buxus* sp., UK	JQ647445	
IMI 311153	*Pseudonectria buxi*	*Buxus sempervirens*, France	JQ693164	
IMI 322095	*Verticillium dahliae*	*Alectryon conaceum*, UK	JQ647437	JQ724138
IMI 364520	*Verticillium dahliae*	*Prunus persica*, Yugoslavia	JQ647438	JQ724137
IMI 62131	*Verticillium nonalfalfae*	*Lycopersicon esculentum*, UK	JQ647435	JQ724134
IMI 67913	*Volutella ciliata*	*Pisum sativum*, UK	JQ647447	
IMI 74420	*Volutella ciliata*	unknown host, Germany	JQ693167	
IMI 341382	*Volutella ciliata*	*Sclerotinia sclerotiorum*, locality unknown	JQ693166	
IMI 341383	*Volutella ciliata*	*Sclerotinia sclerotiorum*, locality unknown	JQ647446	
IMI 332191	*Volutella consors*	unknown source, India	JQ693163	
IMI 92688	*Volutella lini*	unknown host, India	JQ647452	
IMI 224502	*Volutella lini*	unknown host, India	JQ693169	
IMI 136704	*Volutella ramkumarii*	Submerged petioles, Papua New Guinea	JQ647453	

2009; Sigma Genosys, UK). PCR was undertaken in a ThermoHybaid PCR Express thermal cycler (Thermo-Hybaid, UK) using a reaction mix containing 3 pmoles of each primer, 1 µl of template DNA solution and 10 µl of MegaMix-Royal (Microzone Ltd, UK) containing optimised mixture of *Taq* polymerase, anti-*Taq* polymerase monoclonal antibodies in 2 × Reaction Buffer (6 mM $MgCl_2$) with 400 µM dNTPs made up to a final volume of 20 µl with sterilised ultrapure H_2O. Amplification conditions were: 95°C for 5 min followed by 30 cycles of 30 s at 95°C, 30 s at 50°C, 45 s at 72°C, followed by 5 min at 72°C and hold at 10°C.

Aliquots (4 µl) of amplification products were assessed for quality by gel electrophoresis using 1.5 % Seakem LE agarose (BMA, UK) for 2 h at 5V cm^{-1} in half-

strength Tris-Borate-EDTA buffer (*i.e.*, 0.5 × TBE buffer; 45 mM Tris; 45 mM Boric acid; 1.25mM EDTA, pH 7.5, see Sambrook et al. 1989) containing 5 µl of SafeView Nucleic Acid Stain (NBS Biologicals Ltd, UK) per 100 ml of buffer. Gel images were captured by using the U:Genius gel documentation system (Syngene, UK) and stored as TIFF bitmaps for later use.

Remaining unused PCR products were purified with the microCLEAN PCR Purification Kit (Microzone Ltd, UK) following the manufacturer's instructions. The purified PCR products were utilised in sequencing reactions undertaken in a Primus 96 plus thermal cycler (MWG-BIOTECH AG, Germany) by using BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems) with primer TW81 (as above). Sequencing conditions were: 96°C for 1 min followed by 25 cycles of 20 s at 96°C, 10 s at 50°C, 4 min at 60°C (ramp rate: 1°C s^{-1}). Excess unincorporated BigDye was removed with DyeEx 2.0 affinity columns (Qiagen Ltd., UK) according to the manufacturer's instructions and the sequencing reaction products were suspended in HiDi Formamide (Applied Biosystems, UK). These products were separated on a capillary array 3130 Genetic Analyser (Applied Biosystems, UK).

Sequence trace files were first assessed for quality using Sequencing Analysis Software v5.2 Patch 2 (Applied Biosystems, UK) and then checked manually using the software package Chromas v2.23 (Technelysium, Australia) and exported as text files. Sequences were deposited in GenBank and Accession numbers obtained (see Table 1).

Sequences of studied species were aligned using the default parameters of Clustal W (Thompson et al. 1994), then optimised manually using the CLUSTALW plug-in of MEGA5 (Tamura et al. 2011). Phylogenetic inferences were made from Neighbour Joining trees constructed from Maximum Likelihood in Mega5. Branch support was estimated by bootstrap analysis (1000 replicates). Accession numbers of rDNA ITS and GAPDH sequences derived from the public databases are cited on the phylogenetic trees.

Results

Pathogenicity

Besides *Cicer arietinum* (from which the tester strain CP2035 (**IMI** 332702) was isolated), the following leguminous plants were found to function as hosts after experimental inoculation: *Glycine max*, *Phaseolus vulgaris*, *Pisum sativum*, *Vicia faba* and *Vigna unguiculata*. The main symptoms were evident as brown to black lesions on the lower stem parts, within which acervuli develop. Leaves also showed similar symptoms in all species, in addition to chlorosis. The symptoms were very similar to those caused by *Ascochyta rabiei,* a well-known and commonly occurring pathogen causing ascochyta blight on chickpea.

Taxonomy

***Lectera* P.F. Cannon, gen. nov.**
http://species-id.net/wiki/Lectera
Mycobank: MB 550041

Etymology. Named after Dr. Hannibal Lecter (Harris 1988), another aggressive organism with a liking for fava beans (*Vicia faba*).

Conidiomata intermediate between sporodochia and acervuli, erumpent through host tissuesand without a clear upper wall, hemispherical to ± globose, pink or flesh-coloured, accompanied by dark brown septate tapering, primarily marginal, setae. Conidia formed from hardly modified conidiogenous cells, hyaline, aseptate, slightly curved, smooth, fusiform with pointed ends. Appressoria formed after conidial germination, dark brown, round to ovate with smooth margins.

Type species. *Lectera colletotrichoides* (Chilton) P.F. Cannon

***Lectera colletotrichoides* (Chilton) P.F. Cannon, comb. nov.**
Mycobank: MB 550042

Volutella colletotrichoides Chilton, *Mycologia* 46: 801 (1954), basionym.
Volutella colletotrichoides var. *setosa* Chilton, 46: 801 (1954).

Description. Conidiomata formed at the apex of a short peg-like vegetative structure that is erumpent through host tissues, 80–350 µm diam, cushion-shaped to almost globose, with a compact palisade of conidiogenous cells usually surrounded by setae. Setae variably produced, with some conidiomata dark grey in coloration due to copious setae while others are pale pink or flesh-coloured with few or no setae; 50–130 µm in length, 3–6 µm in diam at the base, gradually tapering, golden to dark brown, smooth or sparsely verrucose, 2- to 3-septate, the apex acute. Conidiogenous cells 15–32 × 3–5 µm, cylindrical or slightly tapering with the apex rounded, proliferating percurrently with inconspicuous periclinal thickening and sometimes a minute collarette. Conidia inoculated onto *Medicago sativa* stem (6.5-) 7–10 (-11.5) [mean 8.35 µm, sd 0.73, n = 140] × 2.5–3 (-3.5) µm [mean 2.67 µm, sd 0.24, n = 140], mean length/width ratio 3.14: 1, cylindrical to cylindric-fusiform or navicular, the ends acute, slightly usually inaequilateral with one longitudinal face ± flat, hyaline, aseptate, smooth-walled, without a gelatinous sheath or appendages. Cultures on PCA and PDA at 25°C under alternating daylight/near UV growing moderately slowly, reaching 25–30 mm after 7 d, bright orange with a waxy appearance, the central part becoming brownish after 14 d, aerial mycelium poorly developed. Conidiomata absent or poorly developed, with setae fewer, narrower, shorter and less pigmented than in colonies on host tissue, and conidiogenous cells often formed singly at the apex of vegetative hyphae. Conidia 6.5–9 (-10.5) [mean 7.41 µm, sd 0.68, n = 100] × 2–3 µm [mean 2.43 µm, sd 0.22, n

= 100], mean length/width ratio 3.07: 1, similar in appearance to those formed after inoculation on *Medicago sativa* stems. Appressoria 4.5–8 × 4–6.5 µm, circular to ovate with entire margins, dark brown. Sclerotia not observed.

Typification. USA: Iowa, Ames, on stems of *Medicago sativa,* Oct. 1954, J. Chilton (ISC 217496, **lectotype, here designated**; K(M) 176269, **isolectotype** of *Volutella colletotrichoides*). **USA**: Iowa, Ames, on stems of *Medicago sativa,* Oct. 1954, J. Chilton (ISC 217482, **lectotype, here designated**; K(M) 176270, **isolectotype** of *Volutella colletotrichoides* var. *setosa*). **Other authentic material**: same collection data, ISC 217488, ISC 217497 and ISC 217498 – syntypes of *V. colletotrichoides*; same collection data, ISC 217483 and ISC 217484 – syntypes of *V. colletotrichoides* var. *setosa*.

Host species. Associated with *Asteraceae* (*Xanthium spinosum*), *Fabaceae* (*Cicer arietinum*, *Glycine max*, *Lotus corniculatus* [Chilton, 1954], *Medicago falcata* [Chilton, 1954], *M. sativa*, *Phaseolus vulgaris*, *Pisum sativum*, *Senna sophera*, *Trifolium hybridum* [Chilton, 1954], *T. pratense* [Chilton, 1954], *T. subterraneum*, *Vicia faba* and *Vigna unguiculata*), *Lamiaceae* (*Tectona grandis*), *Poaceae* (*Agrostis stolonifera*, *Hordeum vulgare*, *Triticum* sp. and *Zea mays*), *Solanaceae* (*Capsicum annuum*) and *Violaceae* (*Viola* sp.).

Distribution. Africa (Democratic Republic of the Congo, Egypt, Ethiopia, Morocco, Nigeria, South Africa [http://www.cbs.knaw.nl/], Zimbabwe [unpublished IMI record without voucher material]). Asia (India, Kuwait [unpublished IMI record without voucher material], Turkey [Eken et al. 2002]). Australasia (Australia, New Zealand). Europe (United Kingdom). North America (USA: Iowa, presumed introduced and now eradicated). South America (Argentina, Brazil).

Interactions. Strains of *Lectera colletotrichoides* have been demonstrated to be pathogenic towards a range of *Fabaceae* species, but they are also commonly found associated with plants from other families and isolated from soil and plant litter. It also grows well in standard agar culture. It therefore can be presumed to exist (and probably grow actively) as a saprobe, and it is possible that the non-legume isolates originate from soils used to grow legumes in rotation.

Conservation assessment. The species as currently circumscribed has not been reported since 2002 but is very widely distributed, is associated with a wide range of plant taxa and apparently can exist as a saprobe in soil and leaf litter without a direct plant association. It does not appear to be an economically important pathogen except perhaps in limited circumstances. However, as there is a high risk of confusing disease symptoms of *Lectera colletotrichoides* with those caused by *Ascochyta rabiei*, the economic impact of *Lectera colletotrichoides* may be underestimated. It may be sensitive to agricultural pesticides, but is unlikely to face major threats from specific eradication measures. Its conservation status (Dahlberg and Mueller 2011) must be considered as Data Deficient, but is probably of Least Concern.

Specimens examined. Africa: **DRC**: Mulungu, causing leaf spot of *Glycine soja*, comm. 28 Mar. 1978, *D.J. Allen* (IMI 226829a). **Egypt**: Ismailia, Salheia, isol. ex *Cicer arietinum*, 1988, *M. Askar* CP2035 (IMI 332702). **Ethiopia**: unlocalized, isol. ex seed of *Phaseolus vulgaris*, undated, *s. coll.* (IMI 366179). **Morocco**, unlocalized, isol. ex *Capsicum annuum*, Feb. 1986, *Hasmi* (IMI 303685). **Nigeria**: unlocalized, isol. ex

Vigna sinensis, comm. 22 May 1972, *R. Williams* 4150 (IMI 166385); same data, *R. Williams* 4152 (IMI 166394). **Asia**: **India**: Rajupur, Mirzapur, on and isol. ex *Senna sophera*, Apr. 1982, *S.N.P. Chaurasia* SNPC-5 (IMI 269185); Jabalpur, isol. ex leaf litter of *Tectona grandis*, comm. 5 Feb. 1986, *Jamaluddin* (IMI 265505). **Australasia**: **Australia**: New South Wales, unlocalized, isol. ex *Agrostis stolonifera*, comm. 26 Jan. 1983, *G. Schultz* (IMI 275263); Western Australia: Esperance, isol. ex *Trifolium subterraneum*, comm. 13 Jul. 1972, *R.F. Doepel* (IMI 167533); **New Zealand**: Wellington, isol. ex *Hordeum vulgare*, comm. 11 Apr. 1980, *L. Chong* 18P14 (IMI 247318). **Europe**: **United Kingdom**: Kent, nr Ashford, Wye College, comm. 24 Oct. 1979, *D.W. Parry* 6 (IMI 242382); same locality, isol. ex *Zea mays*, comm. 24 Oct. 1979, *D.W. Parry* 11 (IMI 242387). **South America**: **Argentina**: Córdoba, San Francisco, Monte Cristo, isol. ex stem of *Xanthium spinosum*, 1995, *s. coll.* (IMI 368065). **Brazil**: unlocalized, isol. ex soil, comm. 18 Feb. 1982, *J. Diehl & E. Reis* 46/81 (IMI 265740).

***Lectera longa* P.F. Cannon, sp. nov.**
http://species-id.net/wiki/Lectera_longa
Mycobank: MB 550043

Description. Differs from *Lectera colletotrichoides* by its longer conidia (7.8–10 × 2–2.5 µm; mean 8.87 µm, sd 0.56, n=20) with a mean length/breadth ratio of 3.98: 1, with three short insertions and a single substitution in the ITS sequence.

Typification: Australia. Western Australia: Nedlands, isol. ex *Triticum* sp., 25 Jan. 1974, *K. Sivasithamparam* 530 (IMI 181698) – holotype of *Lectera longa* (dried specimen) and associated living culture.

Host species. Associated with *Triticum* sp. (*Poaceae*).

Distribution. Only definitely known from the type locality.

Interactions. No details are known; the data associated with the type do not indicate whether the fungus was thought to be pathogenic.

Conservation assessment. The species is only known from a single collection made in 1974, though a strain isolated from *Viola* sp. and identified as *Volutella colletotrichoides* with an identical ITS sequence (AJ301962) was deposited in the BBLF culture collection as BBA 71246. Its geographical origin is unknown, and the status of the living culture is uncertain. Its conservation status (Dahlberg and Mueller 2011) must be considered as Data Deficient, but is potentially Critically Endangered.

Discussion

The genus *Volutella* is poorly researched and no modern monograph is available, but some of the more well-known species, including the type *V. ciliata*, were included in a recent phylogenetic study of *Fusarium*-like fungi (Gräfenhan et al. 2011). This work demonstrated that *Volutella* (as represented by its type) occupies a distinct clade within

the *Nectriaceae*, with *Chaetopsina* as sister group and *Pseudonectria buxi* (the anamorph of which was at one time ascribed to *Volutella*) also related. *Volutella* in its currently understood sense is clearly polyphyletic (Seifert et al. 2011), with many of its constituent species referable to other genera.

Lectera has brightly coloured sporodochia surrounded by brown setae (those in true *Volutella* species are hyaline). Other sporodochial genera with these characteristics include *Kutilakesa*, *Sarcopodium* and *Actinostilbe* (Seifert et al. 2011). The taxonomic affinities of the type of *Kutilakesa* are currently unclear (no sequences are available), but the only other species in that genus has a *Nectriella* teleomorph (Alfieri and Samuels 1979) and is therefore likely to belong to the *Hypocreales*. The type of *Sarcopodium* belongs to the *Lanatonectria* clade (*Nectriaceae*, *Hypocreales*) according to Summerbell et al. (2011). Both *Kutilakesa* and *Sarcopodium* have flexuous verruculose setae with rounded apices, in contrast to *Lectera*, where they are straight, hardly ornamented and tapering to an acute tip. On one occasion, a teleomorphic fungus with thin-walled asci and aseptate ascospores was observed on old wilted plant material infected by *L. colletotrichoides*, but the observation could not be repeated and we are not confident that the two fungi are genetically connected.

One other species of *Volutella* has orange sporodochia surrounded by brown tapering setae, in common with *Lectera colletotrichoides*, *V. melaloma* Berk. & Br. which was described from dead leaves of *Carex* in the UK (Berkeley and Broome 1850). It is possible that this species is congeneric with *L. colletotrichoides*, but it differs in a number of important characters: the conidiogenous cells are thicker-walled and do not taper, the setae are longer, more closely septate and smooth-walled, and the conidia are significantly larger and more tapered, measuring 15–18 × 3.5–4.5 µm. No recently collected material is available for sequencing.

The ITS sequences generated in this study strongly suggest that *Lectera* has affinities with the *Plectosphaerellaceae*, and may be a sister group to *Verticillium* (Fig. 1), although this relationship only receives weak bootstrap support. That systematic relationship was previously noted incidentally by Réblová and Seifert (2004), while researching into the phylogeny of *Conioscypha* and its relatives. Many fungi in these groups are wide-spectrum soil-borne plant pathogens, in common with *Lectera*. The *Plectosphaerellaceae* includes a series of *Acremonium*-like fungi as well as *Plectosphaerella*, *Verticillium*, *Gibellulopsis* and *Musicillium* (Zare et al. 2004, 2007). *Verticillium* has recently been monographed using multigene phylogenetic data (Inderbitzin et al. 2011), but its generic position was not assessed. Based on LSU and SSU rDNA data, Réblová et al. (2011) considered the *Plectosphaerellaceae* to be well-defined, and a sister group with the *Glomerellales* to the *Microascales*, an order containing important plant pathogens such as *Ceratocystis*.

Lectera colletotrichoides contains two distinct ITS phylotypes, IMI 265740 differing from the other strains sequenced by only a single base pair. The ITS sequence of *L. longa* differs from *L. colletotrichoides* by three short insertions and a single substitution, and this combined with differences in conidial length and lengh/width ratio justifies its separation. The distinction between the two species is supported by GAPDH sequences also (see fig. 3). The gene fragment sequenced here is a ~200 bp intron in the glyceraldehyde 3-phosphate dehydrogenase gene, used for phylogenetic studies of

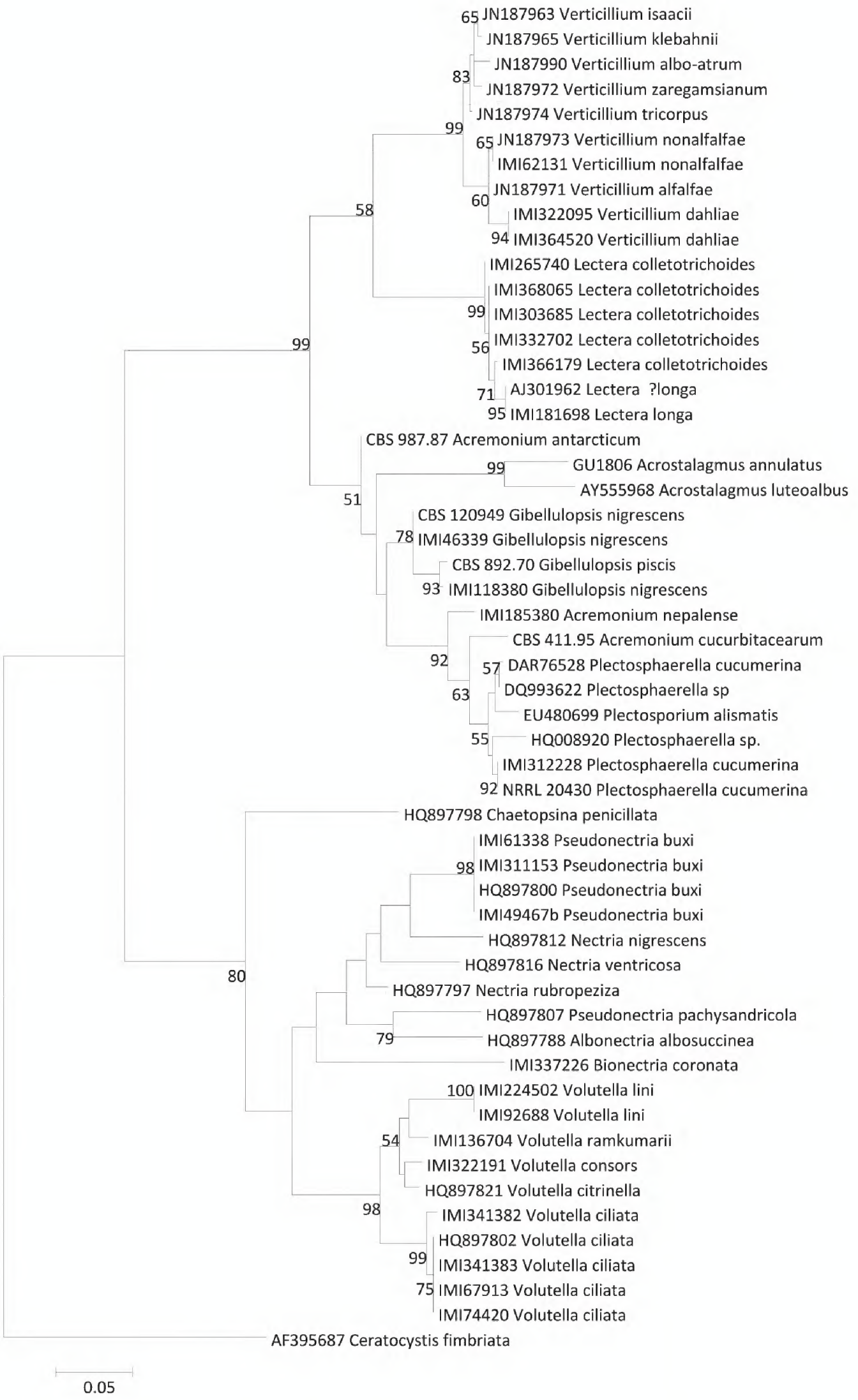

Figure 1. ML ITS phyogram showing the phylogenetic position of *Lectera* species.

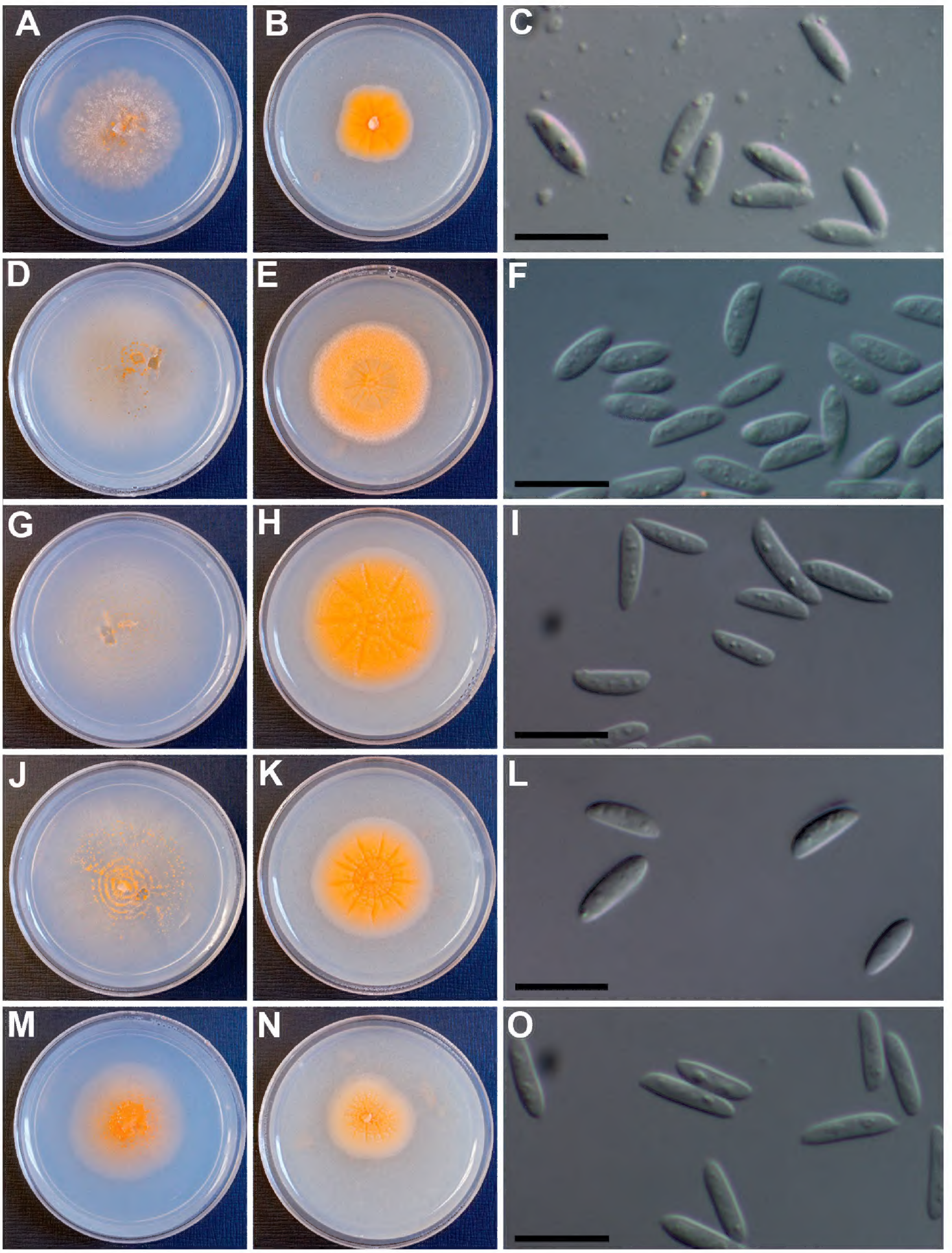

Figure 2. *Lectera* species. **A–L** *L. colletotrichoides* **A–C** IMI 166394 **D–F**, IMI 265740 **G–I** IMI 303685 **J–L** IMI 368065 **M–O** *L. longa* (IMI 181698) **A, D, G, J, M** colonies on PCA agar after 7 d **B, E, H, K, N** colonies on PDA agar after 7 d **C, F, I, L, O**: conidia mounted in lactic acid: bar = 10 μm.

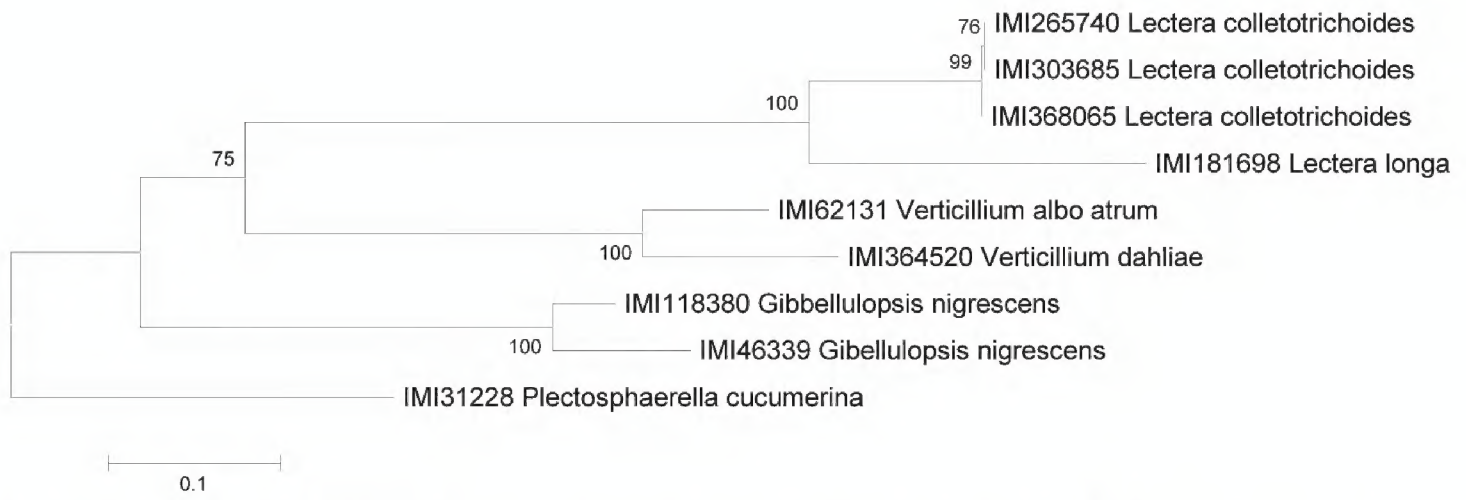

Figure 3. ML tree of GAPDH sequences, of a subset of the strains used for the ITS sequence set.

Colletotrichum by Damm et al. (2009) and Guerber et al. (2003), not the same fragment used for *Verticillium* by Inderbitzin et al. (2011).

The wide distribution of *Lectera colletotrichoides* and its association with agricultural ecosystems make it difficult to assess its geographical origin, although its apparent preference for dry-land legumes might indicate an evolutionary history centred on the Near East. However, judging from environmental sequencing studies, it (or a closely related species) does seem to be present in soils in SE USA (Wu et al. 1997, Jackson 2010), suggesting that its distribution in that country is not as restricted as indicated by Chilton (1954).

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